THE STANDARDIZATION OF ANTIHOG-CHOLERA SERUM *

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The use of antihog-cholera serum is of great importance to the live-stock industries. Since its discovery by Dorset, McBride and Niles in 1908, its use has constantly increased. The potency of the antihog-cholera serum has been tested in the United States by administering serum to susceptible pigs and inoculating them with virulent blood, at the same time giving two or more pigs the same dose of virulent blood alone. If the pigs receiving both serum and virulent blood survive, and those receiving the virus only readily succumb, the serum is declared potent and fit for use in the simultaneous method in doses slightly larger than those used in the test.

Our object has been to ascertain if the various sources of error, affecting the methods of standardization of antihog-cholera serum, may be reduced or eliminated.

The first experiment was carried on to discover whether the hog-cholera serum retained its potency when dried. A quantity of serum was dried in a Faust dryer at a temperature below 40 C. After about two weeks a portion was redissolved in sterile water in such a manner that it was approximately restored to its original volume, and it was then administered intramuscularly to 10 pigs. Each pig in the series was given 2 c.c. of fixed virus of a strain obtained from Dr. John Reichel. The doses of serum were between the limits of 0.05 to 0.4 c.c. per pound, as suggested by Fitzgerald and Fischer. The results of the test are summarized in Table 1.

It is seen that up to the dose of 0.3 c.c. per pound a part of the pigs sickened, and that at 0.4 c.c. all of the pigs were protected. Throughout the remainder of this paper the dose of serum which protects all of the pigs from visible symptoms of disease and from continued temperatures above 104.4 F. will be called the P + dose, the dose which just fails to protect will be termed the P — dose. In these experiments

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TABLE 1

RESULTS OF EXPERIMENTS OF KC CLEAR SERUM AND VIRUS (PIG 7)

No. of Pig	Dose of Serum per Pound	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
34	0.5 c.c.	7	107.2	Died in 11 days
35	0.5 c.c.	6	107.3	Died in 9 days
		Average 6.5		
36	0.1 c.c.	8	106.0	Recovered
37	0.1 c.c.	6	106.1	Died in 7 days
		Average 7		
38	0.2 c.c.	15	106.1	Recovered, symptoms severe
39	0.2 c.c.	2	105.7	Recovered, slight symptoms
		Average 8.5		
40	0.3 c.c.	11	106.4	Killed, chronic
41	0.3 c.c.	0	104.2	Recovered, no symptoms
		Average 5.5		
42	0.4 c.c.	0	104.4	Recovered, no symptoms
..	0.4 c.c.	1	104.7	Recovered, no symptoms
		Average 0.5		

The dose of virus was 2 c.c. in each case.

hogs with temperatures of below 104.5 F. have not usually shown many symptoms, except some cases which tended to become chronic. Therefore, 104.5 F. was arbitrarily chosen as the line of demarcation

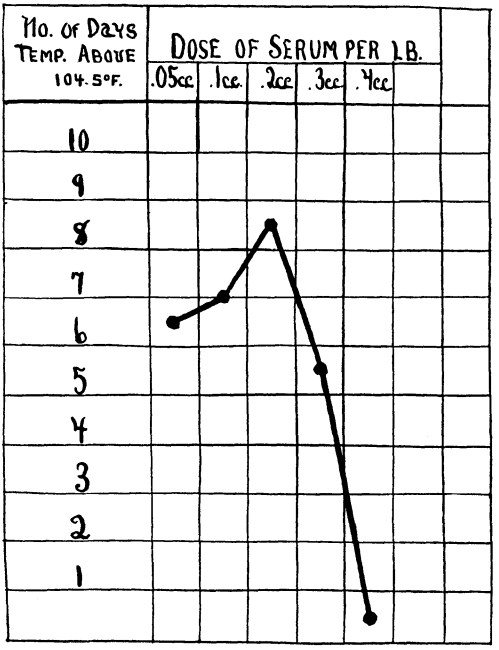

Fig. 1.—The effect of the quantity of serum on the length of time the temperature remained above 104.5 F.

between a slight and definite febrile reaction following the vaccination. The exact temperature chosen does not affect the magnitude of the P + and the P — doses. Considerable attention will be given in the future to establishing the best temperature. In these tests, 104.5 F., however, will be used throughout. Since the data are comparative, the exact point chosen theoretically as well as practically does not affect the accuracy of the results. The pigs receiving doses of serum of the same size formed a group. The number of days' duration of elevated temperature for each group was averaged. When these averages are considered in place of the individual records, considerable regularity is seen in the results. The significance is seen when the doses of serum are plotted on the horizontal axis and the number of days on the vertical (Chart 1).

EFFECT OF DESICCATION AND CENTRIFUGALIZATION

The next test carried out was undertaken to determine whether the serum lost strength in desiccation; also to ascertain if the removal of the red blood-cells affected the magnitude of the P + dose.

Twenty-four hogs were bled from the tail into a covered receptacle. After defibrination 25 per cent. of the blood was preserved with 1 per cent. chloro-

TABLE 2

RESULTS OF EXPERIMENTS WITH WHOLE SERUM AND FIXED VIRUS (PIG 65)

No. of Pig	Dose of Serum per Pound	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
82	0.05 c.c.	5	105.8	Recovered
97	0.05 c.c.	5	104.8	Recovered, slight symptoms
117	0.05 c.c.	5	108.0	Died in 12 days
139	0.05 c.c.	10	107.0	Died in 22 days
		Average 6.2		
83	0.1 c.c.	7	105.2	Recovered
98	0.1 c.c.	5	105.2	Recovered
110	0.1 c.c.	11	106.2	Recovered
124	0.1 c.c.	12	107.0	Recovered
		Average 8.7		
74	0.2 c.c.	7	104.7	Recovered
87	0.2 c.c.	9	107.6	Died in 13 days
112	0.2 c.c.	13	107.0	Recovered
113	0.2 c.c.	3	105.2	Recovered
		Average 8, —P—		
73	0.3 c.c.	3	104.8	Recovered
84	0.3 c.c.	5	105.4	Recovered
120	0.3 c.c.	1	104.7	Recovered
129	0.3 c.c.	3	105.2	Recovered
		Average 3, —P+		
75	0.4 c.c.	1	104.6	Recovered
119	0.4 c.c.	0	103.2	Recovered
103	0.4 c.c.	6	105.3	Recovered
134	0.4 c.c.	4	104.3	Recovered
		Average 2.75		

The dose of virus was 2 c.c. in each case.

TABLE 3

RESULTS OF EXPERIMENTS WITH CENTRIFUGATED SERUM AND FIXED VIRUS (PIG 65)

No. of Pig	Dose of Serum per Pound	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
66	0.05 c.c.	5	106.1	Recovered
155	0.05 c.c.	13	107.7	Recovered
111	0.05 c.c.	5	106.2	Died in 21 days
138	0.05 c.c.	8	107.0	Died in 31 days
		Average 7.7		
86	0.1 c.c.	8	106.0	Died in 29 days
99	0.1 c.c.	5	104.7	Recovered
105	0.1 c.c.	9	106.9	Recovered
123	0.1 c.c.	13	107.5	Died in 21 days
		Average 8.7,—P—		
71	0.2 c.c.	3	105.1	Recovered
132	0.2 c.c.	2	104.8	Recovered
93	0.2 c.c.	1	105.0	Recovered
106	0.2 c.c.	2	104.7	Recovered
		Average 2, —P+		
80	0.3 c.c.	1	104.8	Recovered
121	0.3 c.c.	5	105.2	Recovered
118	0.3 c.c.	1	104.8	Recovered
126	0.3 c.c.	0	104.4	Recovered
		Average 1.7		
68	0.4 c.c.	3	105.1	Recovered
76	0.4 c.c.	2	104.8	Recovered
109	0.4 c.c.	0	104.0	Recovered
133	0.4 c.c.	0	104.2	Recovered
		Average 1.2		

The dose of virus was 2 c.c. in each case.

TABLE 4

RESULTS OF EXPERIMENTS WITH DESICCATED SERUM AND FIXED VIRUS (PIG 65)

No. of Pig	Dose of Serum per Pound	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
67	0.05 c.c.	13	107.8	Died in 16 days
91	0.05 c.c.	12	105.9	Recovered
101	0.05 c.c.	10	105.8	Recovered
137	0.05 c.c.	14	107.7	Died in 21 days
		Average 12		
102	0.1 c.c.	9	106.7	Recovered
122	0.1 c.c.	7	106.4	Recovered
81	0.1 c.c.	3	105.4	Recovered
96	0.1 c.c.	6	105.9	Recovered
		Average 6.2—P—		
85	0.2 c.c.	0	104.1	Recovered
92	0.2 c.c.	1	104.6	Recovered
100	0.2 c.c.	1	104.5	Recovered
128	0.2 c.c.	5	105.6	Recovered
		Average 1.7 —P+		
72	0.3 c.c.	5	104.8	Recovered
135	0.3 c.c.	3	105.1	Recovered
116	0.3 c.c.	4	106.3	Recovered
125	0.3 c.c.	2	104.8	Recovered
		Average 3.3		
77	0.4 c.c.	0	104.0	Recovered
70	0.4 c.c.	1	104.6	Recovered
115	0.4 c.c.	2	105.0	Recovered
..	0.4 c.c.	3	105.2	Recovered
		Average 1.5		

In each case the dose of virus was 2 c.c.

TABLE 5

RESULTS OF EXPERIMENTS WITH WASHED CORPUSCLES AND FIXED VIRUS (PIG 65)

No. of Pig	Dose of Serum per Pound	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
88	30 c.c.	9	107.0	Died in 12 days
136	30 c.c.	9	107.0	Killed moribund in 10 days
94	50 c.c.	8	106.7	Died in 10 days
75	75 c.c.	4	106.8	Recovered

In each case the dose of virus was 2 c.c.

form and saved without further treatment. The remaining 75 per cent. of the blood was freed from corpuscles by centrifugalization. After mixing the various lots of centrifugings together in a large enameled kettle about 1 liter was bottled and the remainder desiccated. In addition to this a certain percentage of the total quantity of red blood-cells from each bleeding was twice washed with sterile salt solution. Defibrinated blood (whole serum), corpuscle-free serum, desiccated corpuscle-free serum, and washed red blood-cells were obtained separately by these procedures. They were all from the same source and, hence, suitable for comparison. Seventy-four pigs were used in the test. Complete series, with four pigs for doses of each size, were carried out with the whole serum, the centrifuged serum, and the desiccated, centrifuged serum. Four pigs received the washed corpuscles. All pigs treated received 2 c.c. of fixed virus and eight check pigs received the virus alone. The results of these tests are summarized in Tables 2, 3, 4, 5 and 6.

These tables show that the desiccated and the centrifuged sera were approximately the same strength, as their P + and P — doses had the same magnitude. The whole serum was distinctly weaker, inasmuch as its P + dose was 0.3 c.c. per pound. The corpuscles have been found to be 40 per cent. of the entire volume of the whole serum, hence its strength should be 60 percent of the strength of centrifuged serum, assuming, as is shown in Table 5, that the washed corpuscles are practically inert. Since the ratio of the P + doses of the two sera is 2 : 3 the strength of the whole serum would be 66.66 + per cent. that of the centrifuged serum. The theoretical strength of the whole serum is 60 per cent. that of the centrifuged, hence the results are in close agreement. It will be seen from Table 2 that the P + dose of the whole serum is 0.3 c.c. per pound, because between the doses of 0.2 c.c. and 0.3 c.c. the average duration of temperature drops from 8 to 3 days. The highest temperature of any of the pigs on the 0.3 c.c. dose was only 105.4, showing that they were protected from serious sickness.

In Table 3, which deals with the centrifuged serum, the P + dose is 0.2 c.c. per pound, and the P — dose 0.1 c.c. per pound, because here the average drops from 8.7 to 2 days.

In Table 4 the P + dose of the desiccated serum is 0.2 c.c. per pound as evidenced by the average which dropped from 6.2 to 1.7 days. The rise to 3.3 days on the dose of 0.3 c.c. per pound is not significant, since a few days temperature above 104.5 is frequently observed in all doses investigated.

It is noted that 30 c.c. of washed corpuscles, which contained two-thirds of its volume, or 20 c.c. of solid red cells, gave little protection. The 50 c.c. dose gave no protection. In the one instance where the 75 c.c. dose was used, there was apparently a little protection. This may have been the result of the trace of serum left. The corpuscles used in this experiment were washed twice with physiologic salt solution. Beginning with 100 c.c. of the defibrinated blood, which by the hematocrite showed 40 per cent. of corpuscles, it was found that 50 c.c., or five-sixths of the amount of serum, could be removed at the first centrifuging. This left 10 c.c. of serum and 40 c.c. of corpuscles. The original volume was restored with the salt solution, and the corpuscles thrown down again. Five-sixths of the total amount of liquid was removed and hence five-sixths of the remaining 10 c.c. of serum, leaving 40 c.c. of corpuscles and 1.66 c.c. of serum. Repeating the process, five-sixths of the 1.66 c.c. of serum was removed, leaving 0.27 c.c. of serum in the 50 c.c. residue after removing the last wash-water. These figures are, probably, a little low, as there is difficulty in removing all the serum adhering to the red cells. Reference to the tests of serum freed from the corpuscles shows that pure serum is considerably stronger.

TABLE 6

RESULTS OF EXPERIMENTS WITH THE VIRUS (PIG 65)

No. of Pig	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
79	8	106.6	Killed moribund in 12 days
89	8	107.2	Killed moribund in 9 days
95	9	107.5	Killed moribund in 10 days
108	9	108.2	Killed moribund in 11 days
107	13	107.6	Killed moribund in 17 days
154	8	107.8	Killed after 8 days, severely affected
130	7	107.5	Killed after 8 days, severely affected
114	6	108.1	Killed after 9 days, severely affected
	Average 8.5		

In each case the dose of virus was 2 c.c.

The eight pigs used in this experiment all rapidly developed acute hog-cholera. These pigs were selected from the four lots of pigs used in the preceding experiment. Two pigs came from each lot. This demonstrates that the pigs used were susceptible and the virus active.

THE EFFECT OF ALUMINUM ON SERUM

The third series of tests was carried out to ascertain if aluminum is a suitable metal in which to store serum. As antihog-cholera serum is produced in very large amounts there is need of some container in which large quantities may be mixed together. Covered vessels up to 250 gallons capacity may be constructed out of seamless aluminum and so arranged that they may be sterilized by heat. As aluminum is non-toxic and non-corrosive and readily cleaned it would seem to be admirably adapted for this use.

In this test the aluminum was in contact with the serum for twenty-four hours with constant shaking. This is several times longer than the serum would remain in contact with the aluminum in the mixing process.

A quantity of desiccated anti-hog-cholera serum was dissolved and one-half placed in an aluminum flask, and the other half put into a glass flask. Both were shaken for twenty-four hours at room temperature. A quantity of

TABLE 7

RESULTS OF EXPERIMENTS OF STANDARD AND FILTERED VIRUS

No. of Pig	Dose of Serum per Pound	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
292	0.02 c.c.	2	105.4	Recovered
291	0.02 c.c.	3	105.2	Recovered
332	0.02 c.c.	4	106.0	Recovered
		Average 3		
320	0.05 c.c.	7	107.2	Killed moribund in 10 days
304	0.05 c.c.	0	103.8	Recovered
305	0.05 c.c.	0	103.6	Recovered
		Average 2.33		
315	0.1 c.c.	3	105.8	Recovered
312	0.1 c.c.	10	107.0	Killed moribund in 11 days
318	0.1 c.c.	6	107.0	Still sick at end of 3 weeks
		Average 6.33		
336	0.2 c.c.	2	105.2	Recovered
339	0.2 c.c.	1	104.8	Recovered
342	0.2 c.c.	0	103.6	Recovered
		Average 1 P+		
345	0.3 c.c.	3	104.9	Recovered
348	0.3 c.c.	1	104.9	Recovered
351	0.3 c.c.	3	106.2	Recovered
		Average 2.33		
354	0.4 c.c.	0	103.6	Recovered
357	0.4 c.c.	0	104.4	Recovered
360	0.4 c.c.	0	104.2	Recovered
		Average 0		

In each case the dose of filtered virus was 15 c.c.

TABLE 8

RESULTS OF EXPERIMENT WITH ALUMINUM TREATED SERUM AND FILTERED VIRUS

No. of Pig	Dose of Serum per Pound	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
301	0.02 c.c.	9	107.0	Killed moribund in 11 days
324	0.02 c.c.	8	107.6	Killed moribund in 10 days
296	0.02 c.c.	0	103.2	No symptoms
		Average 5.66		
298	0.05 c.c.	7	105.6	Recovered
314	0.05 c.c.	1	105.6	Recovered
319	0.05 c.c.	10	107.2	Died in 12 days
		Average 6		
306	0.1 c.c.	9	107.4	Died in 21 days
303	0.1 c.c.	0	104.4	Recovered
325	0.1 c.c.	0	107.0	Died in 11 days
		Average 6 P —		
337	0.2 c.c.	3	104.6	Recovered
340	0.2 c.c.	0	104.4	Recovered
343	0.2 c.c.	1	105.0	Recovered
		Average 1.33 P+		
346	0.3 c.c.	1	104.7	Recovered
349	0.3 c.c.	0	103.7	Recovered
352	0.3 c.c.	0	104.3	Recovered
		Average 0.33		
355	0.4 c.c.	1	105.6	Recovered
358	0.4 c.c.	0	104.0	Recovered
361	0.4 c.c.	2	105.0	Recovered
		Average 1		

In each case the dose of filtered virus was 15 c.c.

TABLE 9

RESULTS OF EXPERIMENTS WITH K. C. SERUM AND FILTERED VIRUS

No. of Pig	Dose of Serum per Pound	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
308	0.02 c.c.	0	104.3	Recovered
328	0.02 c.c.	6	106.0	Died in 9 days
333	0.02 c.c.	10	107.0	Killed moribund in 10 days
		Average 5.3		
313	0.06 c.c.	10	107.0	Killed moribund in 13 days
329	0.06 c.c.	7	107.0	Killed moribund in 10 days
317	0.06 c.c.	7	108.0	Killed moribund in 9 days
		Average 8		
293	0.01 c.c.	9	105.6	Recovered
322	0.01 c.c.	6	107.0	Died in 11 days
310	0.01 c.c.	5	107.0	Still somewhat sick at end of 21 days
		Average 6.66		
338	0.2 c.c.	4	105.2	Recovered
341	0.2 c.c.	5	105.8	Recovered
344	0.2 c.c.	9	107.4	Died in 11 days
		Average 6		
347	0.3 c.c.	1	105.6	Recovered
350	0.3 c.c.	6	106.2	Recovered
353	0.3 c.c.	0	104.0	Recovered
		Average 2.33		
356	0.4 c.c.	2	105.0	Recovered
359	0.4 c.c.	0	104.0	Recovered
363	0.4 c.c.	4	105.5	Recovered
		Average 2		

serum from another source was dried in a Faust dryer. The three sera were injected into three series of test pigs on the same day against the same virus. Weights were balanced, but owing to an error it was impossible to balance origin of pigs. The results are set forth in Tables 7, 8, 9 and 10.

TABLE 10

RESULTS OF EXPERIMENTS WITH FILTERED VIRUS

No. of Pig	No. of Days Temperature Above 104.4 F.	Highest Temperature	Results
288	10	106.0	Died in 14 days
316	9	107.2	Killed moribund in 10 days
311	7	107.6	Died in 8 days
	Average 8.66		

In each case in both Tables 9 and 10 the dose of filtered virus was 15 c.c.

Tables 7 and 8 show that the P + dose of the serum is the same, 0.2 c.c. per pound, when the serum is kept in glass and when the same serum is treated with aluminum.

Therefore we may conclude that, within the limits of accuracy of the method, the aluminum showed no deleterious action on the serum.

Table 9 shows that the K. C. serum has a P + dose of 0.3 c.c. per pound, and therefore must be used in 50 percent greater doses than the standard serum.

Table 10 shows that the filtered virus used was quite virulent.

PRINCIPLES OF STANDARDIZATION

These experiments indicate that, for scientific purposes at least, considerable accuracy may be attained in the standardization of antihog-cholera serum.

If it were not for the varying strength of the hog-cholera virus and the various grades of susceptibility exhibited toward hog-cholera by pigs of different breeds, ages, weights and sources, the determination of the P + dose might be sufficient standardization for a serum. However, in order to accurately standardize a serum, these variables must be eliminated. In addition to the irregular susceptibility of hogs to natural and artificial infection with hog-cholera, the filterable virus as shown by Uhlenhut varies in strength quite rapidly when stored, and the antihog-cholera serum, in the liquid form, probably gradually loses its strength through the action of light, heat, oxygen and moisture. The effect of these disturbing factors causes a variation in the magnitude of the P + dose of the same serum when the determination

is made on pigs from different sources, or on pigs from the same source with different virus. The exact amount of this variation has not, as yet, been determined. The use of a standard desiccated antiserum gives promise of eliminating these variables.

Ehrlich has shown that antisera, when completely desiccated and stored in high vacuo in a dark, cool place, remain practically unchanged in potency for long periods of time, and that samples of diphtheria antitoxin so preserved, when used as an arbitrary unit, furnish the most reliable method for the standardization of diphtheria antitoxin.

By desiccating antihog-cholera serum in an analogous manner there is every reason to believe that it becomes sufficiently stable to furnish a standard or unit by which the strength of any sample of hog-cholera serum may be more accurately measured, if the test is so arranged that one-half of the pigs are given the standard serum in graduated doses, and the other half receive the serum to be tested in doses of the same size, and all pigs receive the same virus in doses of the same size.

Three or four pigs are used on each sized dose of serum in order that the average may be as accurate as possible. The pigs are obtained from herds in which cholera has not existed, and in which vaccination has not been practiced. They are held in quarantine from ten days to two weeks in pens widely separated from infection. Their origin is noted because the susceptibility of pigs from different origins — farms — varies greatly. On the day of the test they are weighed and divided into squads in such a manner that for every pig of a certain origin used in testing the unknown serum a pig of about the same weight and the same origin, will receive the standard serum in doses of the same size. This principle of balancing weights and origins of pigs is also applied to the selection of pigs for each rate of serum, so that any difference in susceptibility of the pig will balance throughout the test and, hence, this factor will be eliminated, since the same virus has been used on all pigs. In each series the variation in the strength of the virus is likewise eliminated.

When the magnitude of the P + dose of standard serum and the P + dose of the serum to be tested are simultaneously determined as outlined above, the ratio of the P + unknown to the P + standard is independent of the virus used, and largely independent of the susceptibility of the pigs used. More accuracy is attained if large numbers of hogs are used on doses of each size and large numbers of doses tested

between the limits of 0.05 and 0.4 c.c. per pound. The limits of accuracy of the method have not been exactly determined.

Although the standardization of anti-infectious serum can probably never be so simple or so accurate as the standardization of an antitoxic serum against its antitoxin, nevertheless antitoxic sera; e. g., blackleg and swine plague sera (Schweineseuche), are standardized with considerable accuracy. The fact that we are dealing with an ultravisible virus and that we cannot determine its concentration does not seem to unusually complicate the method, as even in those diseases in which we are dealing with a visible organism, which may be raised *in vitro,* the variations in virulency are so great that the determination of the number of organisms injected is not a definite measure of the virulence of the test dose.

A future paper will deal more minutely with the various features of the proposed method of standardization.

CONCLUSIONS

When a series of pigs were inoculated with increasing serum and virus, a point was reached at which the pigs no longer showed more than a transient fever. This dose is the protective dose of serum.

The same serum tested in both the desiccated and non-desiccated forms showed the same strength.

A serum, from which the red blood-cells had been removed, was definitely more potent than the same serum containing red blood-cells.

The potency of the serum was not measurably affected by storage in aluminum for twenty-four hours.